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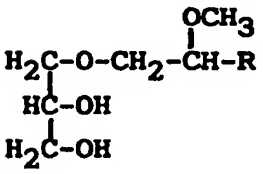
(54) Title: PHARMACEUTICAL COMPOSITION AND USE THEREOF

(57) Abstract

A pharmaceutical composition for the treatment of cancer is provided, containing as an active ingredient at least one 2-methoxy-substituted alkylglycerol of formula (I) wherein R is a hydrogen atom or a straight or branched, saturated or unsaturated alkyl group of from 1 to 24 carbon atoms; and also containing at least one cytostatically active agent. Moreover, the user of 2-methoxy-substituted alkylglycerols according to the above formula in association with a cytostatically active agent in the chemotherapeutic treatment of cancer is provided, as well as a chemotherapeutic method by use of the inventive compositions.



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PHARMACEUTICAL COMPOSITION AND USE THEREOF

The present invention relates to the use of 2-methoxy-substituted alkylglycerols in association with a cytostatically active agent in the chemotherapeutic treatment of cancer.

More specifically the invention relates to the use of 2-methoxy-substituted alkylglycerols in association with a cytostatically active agent in the preparation of a pharmaceutical compostion useful in the chemotherapeutic treatment of cancer, and to the pharmaceutical compositions obtained by such use.

BACKGROUND OF THE INVENTION

There are different ways of classifying malignant diseases, As an example, they can be subdivided into cancers, involving the epithelial cells, and sarcoma, involving the mesenchymal cells. However, this classification of cancers vs. sarcoma is not entirely complete, as there also exist malignant diseases involving e.g. the blood, the lymphoma, the nerv cells of the central nervous system.

The common characteristic of all these malignant diseases is their capability of giving rise to tumours, first locally and then, through metastases, throughout the whole body. The treatment frequently is performed by surgery, followed by radiation therapy and chemotherapy. Often combinations of two or three of any of these methods have been resorted to.

In the chemotherapeutic treatment of tumours, use is made of cytostatic, i.e. cytotoxic, agents. Their utility is based on the fact that the tumour cells multiply much faster than the normal, healthy cells, leading to a greater susceptibility in the tumour cells to the cytotoxic effect of the cytostatic agents. However, the normal cells, especially those having a naturally high division rate, also will be disturbed by the cytostatic agent, sometimes leading severe negative side

effects of the cytostatic agent, which in the best case will be reversed after interruption of the chemotherapy.

The cytostatic agents used in chemotherapy may act by various mechanisms and be of various origins. Thus, the presently known cytostatic agents may be classified into:

- alkylating agents, attacking the cells in the mitosis stage, i.e. the cell division stage, and thereby preferentially the tumour cells;
- antimetabolites, antagonistically acting on the cell meta bolism, which is higher in the faster dividing tumour cells, these then being more affected;
- certain plant alkaloids, used as cell killing agents;
- certain antibiotics having a cytostatic effect through a cell degrading activity;
- hormones and hormone antagonists, acting by various mechanisms, such as by stimulating other cell activity to thereby inhibit the cell division activity, or by acting as a target specific vehicle for another cytostatic agent;
- other sorts of cytostatic agents not falling within the above groups.

From the above it should be apparent that cytostatic agents form a very heterogeneous group, having in common that they damage the cells of the body and that this damage hits the tumour cells harder than the normal, healthy cells. Different cytostatic agents frequently are used in combination with each other to attack the tumour cells in several ways at the same time.

As stated above, however, the use of cytostatic agents frequently brings about undesirable negative side-effects. Indeed, the faster proliferation of the cancer cells, which make them more susceptible to the activity of the cytostatic agent, is a characteristic they share more or less with some of the healthy cells of the body. Thus, e.g. in the bone marrow and in the reproductive organes the cell proliferation is very fast,

making these organs vulnerable to the cytotoxic effects in a chemotherapeutic treatment. Also, in young children the cell division rate is high whereas their hepatic detoxification system is still incomplete.

In view of the drawbacks of the chemotherapy for cancer by use of conventional cytostatic agents, some workers have tried to act by modifying the milieu that the tumour presides in. The findings of i.a. Burns and Spector (Burns, C.P., Spector A.A. "Effects of lipids on cancer therapy." Nutr Rev (1990 Jun) 48(6): 233-40) suggested a potential role for lipid nutrition in cancer therapy. It has been well documented that the fatty acid content of cell membranes, of healthy as well as cancerous cells, can change substantially when the cells are exposed to different types of fat. Thus, in EP A 0 238 198 Wood et al. proposed a cancer treatment by the administration of saturated fatty acids and/or a fatty acid inhibitor of an enzyme converting membraneous saturated fatty acid to unsaturated fatty acid.

It has been shown (Das et al., "Dietary Ether Lipid Incorporation into tissue Plasmalogens of Humans and Rodents.", Lipids (1992) 27:401-405) that dietary ether lipids can be directly utilized by mammals to synthetized membrane alkyl glycerolipids and plasmalogens in most tissues. Several studies have shown that the amount of alkyldiacylglycerols is much higher in neoplastic cells than in normal cells (Spener, F. "Ether Lipids in Clinical Diagnosis and Medical Research." in "Ether Lipids Biochemical and Biomedical Aspects" (eds. Mangold, H.K. and Paltauf F.P.) New York: Academic Press, 1983, 239-259). The amount in tumourous tissue can be 10-100 times higher as compared with normal tissue. The explanation is that the tumourous tissue contains extremely low amounts of ether cleavage enzyme.

The above mentioned German research groups have shown that alkyllysophospholipids can activate macrophages in the bone

marrow. The German researchers believe that the macrophage stimulating effects of alkyllysophospholipids explain the effect of these substances on tumours and tumour spread. Since tumour cells have only a low activity of enzymes which can break down ethers, alkylethers are incorporated into the cell membrane's phospholipids which are then recognized and attacked by the macrophages, having a high activity of ether catabolic enzymes.

Nonetheless, in view of the drawbacks of the conventional cytostatic agents, and in spite of the above cited proposed methods of treating cancer by acting on the milieu of the tumour, there still is a great need for new methods of chemotherapeutic treatment of cancer. The object of the present invention is to provide for such a method by a new pharmaceutical composition applicable therein.

The inventive method is based on the use of a methoxy-substituted alkylglycerol in association with a cytostatically active agent.

Methoxy-substituted alkylglycerols have been shown to inhibit tumour growth in cultured cell lines (US A 4,046,914 (1978) to Hallgren et al.).

Said methoxy-substituted alkylglycerols belong to the family of the so called alkylglycerols (also referred to as glyceryl ethers, glycerol ethers or alkoxy glycerols), represented by the formula CH_2OH-CHOH-CH_2OR, wherein R is a saturated or unstaurated alkyl group, which in the case of the methoxy-substituted alkylglycerols is substituted with a CH_3O- group.

The alkylglycerols have a widespread distribution in nature. They occur, mostly as di-esters of fatty acids, in the lipids of various animal organs and are found for example in bone marrow fat, in the fat of the spleen and liver, in the erythrocytes in the plasma lipids and in breast milk. Alkylgly-

cerols also have been found as the unsaponifiable fraction of many marine oils. The most important source is the liver oil of certain elasmobranch fish. Alkylglycerols occurring in natural fats were first discovered in the unsaponifiable fraction of liver oils from sharks and rays. It was found that shark liver oils contain mainly selachyl alcohol (9-octadecenylglycerol) together with chimyl alcohol (hexadecylglycerol) and batyl alcohol (octadecylglycerol). For a thorough review of the occurrence and characteristics of the alkylglycerols, refer to the above mentioned textbook "Ether Lipids, Biochemical and Biomedical Aspects", as well as to "Ether Lipids, Chemistry and Biology", Ed. Snyder F., Academic Press (1972)).

A great variation in the quantities of alkylglycerols in the liver oils is observed between different species of sharks. For example, according to Hallgren B and Larsson S ("The glyceryl ethers in liver oils of elasmobranch fish" Lipid Research Vol.3, No.1 (January 1962)) the liver oil from Somniosus microcephalus, the Greenland shark, contains 7% of alkylglycerols whereas the liver oil from the so called Seamouse, Chimera monstruosa contains 33% of alkylglycerols.

Within the broader family of naturally derived alkylglycerols, methoxy-substituted alkylglycerols usually account for only a very low percentage, which varies depending on the source of the alkylglycerols. Hallgren et al. in US A 4,046,914 stated that the methoxy-substituted alkylglycerols account for 3% by weight of the alkylglycerol fraction. They furthermore identified these methoxy-substituted alkylglycerols as being mainly (2-methoxy-4-hexadecenyl)-α-glycerylether (or 1-O-(2-methoxy)-4-hexadecenylglycerol), (60% by weight); (2-methoxyhexadecyl)-α-glycerylether (or 1-O-(2-methoxy)hexadecylglycerol), (15% by weight); and (2-methoxy-4-octadecenyl)-α-glycerylether (or 1-O-(2-methoxy)-4-octadecenylglycerol), (20% by weight).

Numerous biological effects have been attributed to the alkyl-

glycerols. They have i.a. been shown to have an immunostimulating effect e.g. on the immunoresponses in pups when given in the diet to lactating rats (Oh and Jadhav, 1994); they also have a beneficial influence on the so-called epidermal growth factor (Brohult A and Brohult S, SE 8705035-7); an anti allergic effect useful in the treatment of i.a. asthma (Brohult A and Brohult S, SE 8800182-1). Moreover, they have been shown to possess a stimulating effect on hematopoiesis, including erythropoiesis, thrombocytosis and granulocytosis (Edlund T, "Protective effect of d,l,α-octadecylglycerol ether in mice given total body x-irradiation", Nature 174:1102 (1954); Linman JW "Hematopoietic effects of glyceryl ethers III. Inactivity of selachyl alcohol", Proc Soc Exp Biol Med 104:703-706 (1960); and Osmond DG et al. "The action of batyl alcohol and selachyl alcohol on the bone marrow of the guinea pig", Acta Hematol 29:180-186 (1963)). These characteristics have been used in conjunction with the observed immunostimulating effect to ameliorate the survival rate in the radiation, radiomimetic and cytostatic tumour therapies (Brohult A H et al. in US A 3,432,602). In said patent document, it is taught that alkylglycerols of not more than 26 carbon atoms, mainly those of 14-24 carbon atoms derived from shark liver oil, cause an activation and stimulation of the defence mechanisms of a person or animal treated, and thus pharmaceutical preparations of such compounds may be used prophylactically or curatively against exposure to harmful radiation. The authors observe that the defense mechanisms of the body are stimulated by said compounds, which however do not show an germicidal effect, being in fact able to stimulate the growth of e.g. Lactobacillus lactis. On the basis of this observation they emit the theory that the beneficial effect of the alkylglycerols are of a systemic nature, which might well be an activation of notably the reticuloendothelial system.

In said patent no mention is made of methoxy-substituted alkylglycerols.

Further documents dealing with the beneficial effects of alkyl glycerols on different cancer forms are "Alkoxyglycerols and their use in radiation treatment" (Brohult A. Acta Radiol 1963, Suppl 223); and "Biochemical effects of alkoxyglycerols and their use in cancer therapy" (Brohult A. et. al. Acta Chem Scand 1970: 24, 730).

Hallgren et al. in US A 4,046,914 teach that the methoxysubstituted alkylglycerols, although chemically and physically very nearly related to the non substituted alkylglycerols, in fact possess characteristics different from those of the latter ones, since, unlike their non substituted counterparts, the methoxy-substituted alkylglycerols have an antibiotic activity against several types of bacteria, and moreover show no effect on the number of red and white blood cells at the concentrations tested. These workers also compared the effect obtained on the growth of transplantated mammary cancer in mice using a total glyceryl ether fraction extracted from shark liver oil, and only the methoxy-substituted alkylglycerols, respectively, and found that while the total extract had a stimulating effect on the growth of the cancer, the methoxy-substituted alkylglycerols showed a growth retarding effect thereupon, and also suppressed the formation of metastases in the lungs. They thus concluded that the methoxy-substituted alkylglycerols differ from the ordinary (non substituted) alkylglycerols in several biological respects.

Hallgren et al. furthermore found that the methoxy-substituted alkylglycerols had an inhibiting effect on the in vitro growth of human cancer cells, namely He La cells. They conclude that "the methoxy-substituted glycerol ethers offer a new type of substances which might be used for inhibiting tumour growth in patients, possibly with other treatments", without, however, specifying any particular treatment.

SUMMARY OF THE INVENTION

The present invention is based on the surprising finding of a pronounced synergistic effect between 2-methoxy-substituted alkylglycerols and conventional cytostatic agents.

Indeed, while both components on their own are capable of showing a cytostatic effect, in association with each other they however give a synergistically enhanced effect, beyond that of a simple addition effect. This synergistic effect also provides for a chemotherapeutic composition having substantially less negative side effects, since it may be used in relatively lower dosages.

Although not wishing to be bound to any theory, it may be suggested that the 2-methoxy-substituted alkylglycerols, in the same way as has been shown for ordinary alkylglycerols, are accumulated in the cell membrane of preferentially the tumour cells (Spener, vide supra). The presence of the methoxy group will however lead to the cell being unable to degrade the 2-methoxy-substituted alkylglycerol, since the substituted compound is not a naturally occurring compound in the human body. The fact that the methoxy group is in the 2-position moreover will lead to a bulkiness which by disturbing the cell membrane structure facilitates the penetration thereof by the cytostatic agent. For this effect to be achieved it is vital that the methoxy group is in the 2-position. Farther away from the glycerol portion, it would be accessible to the acetyl-coenzyme A, and thus would enter the fatty acid metabolism of the cell.

The 2-methoxy-substituted alkylglycerols will thus lead to a better selectivity of the cytostatic agent, by facilitating its entry into the tumour cells. The better selectivity will result in smaller dosages of cytostatic agents being needed to achieve the same effect in a chemotherapeutic therapy of a cancer. By the possibility of using smaller dosages of cytostatic agent and by the better selectivity at the dosages used,

severe negative side effects, such as anaemia, digestive disorders, diminshed fertility, hair loss, and, in children, growth inhibition, may therefore be greatly alleviated.

The present invention thus provides a pharmaceutical composition comprising one or more methoxysubstituted alkylglycerols in association with one or more cytostatic agents, said composition being useful in the chemotherapeutic treatment of cancer.

In another aspect the invention provides the use of methoxy-substituted alkylglycerols in the preparation of a composition comprising also a cytostatic agent for the chemotherapeutic treatment of cancer.

In still another aspect the invention provides the use of methoxysubstituted alkylglycerols in the preparation of a pharmaceutical composition useful to synergistically enhance the efficiency and specificity of one or more cytostatic agents for the chemotherapeutic treatment of cancer.

Finally, the invention also provides a method of chemotherapeutic treatment of cancer either by use of a cytostatically active composition according to the invention, comprising one or more 2-methoxy-substituted alkylglycerols in association with one or more cytostatic agents, or by use of one or more cytostatic agents in conjunction with a composition comprising one or more 2-methoxy-substituted alkylglycerols to synergistically enhance the chemotherapeutic effect of said cytostatic agent(s).

DETAILED DESCRIPTION OF THE INVENTION

1) The 2-methoxy-substituted alkylglycerols

The 2-methoxy-substituted alkylglycerols of use in the invention may be of natural or synthetic origin.

The main natural source of 2-methoxy-substituted alkylglycerols generally is the same as that of the non substituted alkylglycerols, i.e. the liver oil of elasmobranch fish, mainly the Greenland shark (Somniosus microcephalus) and the Seamouse, Chimera monstruosa. There is no strict limitation regarding the source, however, and other fish oils may equally be used, as convenient. There also exist other sources of said compunds, since they occur in the lipids of various animal and human organs, e.g. in the bone marrow, the liver and the spleen. It should be understood that the source of the 2-methoxy-substituted alkoxyglycerols is not critical to the invention, as the compunds of interest may be extracted and purified using the ordinary separation and analysis procedures, known to the person skilled in the art. The distribution of said compounds in nature as well as separation and analysis techniques thereof are extensively disclosed in the above mentioned textbooks, i.e. "Ether Lipids, Biochemical and Biomedical Aspects" and "Ether Lipids, Chemistry and Biology" and are included herein by reference.

The 2-methoxy-substituted alkylglycerols found in the shark liver oil from the Greenland shark are: the 2-methoxy-substituted saturated alkylglycerols , viz. 1-O-(2-methoxy)tetradecylglycerol (14:0), 1-O-(2-methoxy)pentadecylglycerol (15:0), 1-O-(2-methoxy)hexadecylglycerol (16:0), 1-O-(2-methoxy)heptadecylglycerol (17:0), 1-O-(2-methoxy)octadecylglycerol (18:0), 1-O-(2-methoxy)nonadecylglycerol (19:0), 1-O-(2-methoxy)eichosylglycerol (20:0) and 1-O-(2-methoxy)docosylglycerol (22:0); the 2-methoxy-substituted mono-unsaturated alkylglycerols, viz. 1-O-(2-methoxy)-4-hexadecenylglycerol (16:1), 1-O-(2-methoxy)heptadecenylglycerol (17:1), 1-O-(2-methoxy)-4-octadecenylglycerol (18:1), 1-O-(2-methoxy)nonadecenylglycerol (19:1), 1-O-(2-methoxy)eichosenylglycerol (20:1) and 1-O-(2-methoxy)docosenylglycerol (22:1); and the hexa-unsaturated 1-O-(2-methoxy)docosahexaenylglycerol (22:6).

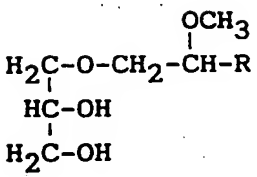
The numbers within brackets after the name of each alkylglycerol is a conventional way of describing the alkyl chain whereby the first number represents the number of carbon atoms in the main chain of the alkylgroup and the second number represents the number of double bindings of said chain.

Whereas the broad range of 2-methoxy-substituted alkylglycerols present in Greenland shark liver oil is given above, in fact the predominant of these are the 1-O-(2-methoxy)hexadecylglycerol (16:0) (15% by weight), 1-O-(2-methoxy)-4-hexadecenylglycerol (16:1) (60% by weight) and the 1-O-(2-methoxy)-4-octadecenylglycerol (18:1) (20% by weight).

The 2-methoxy-substituted alkylglycerol composition of fish liver oils from other species may of course be different from that of e.g. the Greenland shark. However, we believe that the alkyl chain length is of minor importance to the activity of the inventive compositions, as long as the 2-methoxy group is present on the molecule.

For example, an alkyl chain length of 2 to 26 carbon atoms is contemplated, and the alkyl chain could also be a branched chain. Moreover, the number of unsaturations of the alkyl chain is not strictly critical. Thus, the alkyl chain beyond the 2-methoxy group may vary in its chemical structure within quite large limits, provided it does not interfere with the synergistic activity of the molecule. If, practicing the invention, the man of ordinary skill in the art should want to try to find out the usefulness of a given 2-methoxy-substituted alkylglycerol not mentioned herein, this would be a question of routine experimentation well within his capability.

A general formula of preferred 2-methoxy-substituted alkylglycerols according to the invention is given by:



wherein R is a hydrogen atom or a straight or branched chain, saturated or unsaturated alkyl group of from 1 to 24 carbon atoms.

In a specially preferred embodiment of the invention 1-O-(2-methoxy)hexadecylglycerol is used.

The 2-methoxy-substituted alkylglycerols of the invention may also be synthetized, e.g. using the method of Ställberg G (Chemica Scripta 7, 31-41 (1975)).

2) The cytostatic agent

The invention is not limited to any special type of cytostatic agent. Thus, one or more cytostatic agent selected from one or more from any one of the herein above enumerated types may be used.

As an example, the cytostatic agent may be selected from doxorubicin and fluorouracil. The former one belongs to the class of antibiotics having a cytostatic effect, whereas the latter one belongs to the class of the antimetabolites.

3) Administration forms, doses and other ingredients

The pharmaceutical composition of the invention may be administered by the oral route, e.g. as a lipid or oily solution or emulsion of the cytostatic agent in the methoxysubstituted alkylglycerol contained in e.g. a soft gelatin capsule, optionally in combination with other physiologically acceptable lipids as well as with other conventional constituents, such as e.g. preservatives, known to the person skilled in the art.

The pharmaceutical composition of the invention may also be provided as tablets, formed of micellas of the active compounds in an oily vehicle consisting of a pharmaceutically acceptable oil, such as an edible oil, e.g. a vegetable oil.

Moreover, administration may be performed by injection, whereby the composition preferably is in the form of a lipid solution.

In one embodiment of the invention, the cytostatic agent and the 2-methoxysubstituted alkylglycerol, while intented to be taken in combination with each other still is administered as physically separate entities. This has the advantage of an easy variation of e.g. the cytostatic species or the dosage thereof, in case one would want to perform such a variation during the course of a chemotherapeutic treatment.

A useful ratio of the cytostatic agent to the 2-methoxysubstituted alkylglycerol will be from 1:100 to 100:1 on a molar basis. Another useful ratio of the cytostatic agent to the 2-methoxysubstituted alkylglycerol will be from 1:20 to 20:1, on a molar basis. The cytostatic agent to the 2-methoxysubstituted alkylglycerol may also usefully be present in a ratio to each other of from 1:10 to 10:1, on a molar basis. Another useful ratio of the cytostatic agent to the 2-methoxysubstituted alkylglycerol will be from 1:5 to 5:1, on a molar basis. Finally, the cytostatic agent to the 2-methoxysubstituted alkylglycerol will usefully be present in a ratio of from 1:2 to 2:1, on a molar basis.

4) Therapeutic method

Any disease characterized by the presence of tumour cells and susceptible to chemotherapeutic treatment may be subject to treatment by the pharmaceutical composition of the invention. Consequently, the type of malignant disease - cancer or sarcoma or any other type of malignant disease not covered by these terms - is not critical to the possibility of treatment

by use of the pharmaceutical composition of the invention.

The selection of the cytostatic agent and the dosage thereof, as well as the possible combination of the administration of the composition of the invention and any other treatment method, such as by surgery or radiation therapy, will be left to the judgment of the skilled practitioner, having regard to the usual parameters, such as the treated condition, the age, sex and condition of the treated subject etc.

Similarly, the dosage of 2-methoxysubstituted alkylglycerol will of course be subject to variations. However, the dosage will be chosen in relation to the clinical situation as well as the dosage of the cytostatic agent, as stated herein above.

A suitable daily dosage of the 2-methoxysubstituted alkylglycerol may be from 3 to 30 mg, whereas a suitable daily dose of the cytostatic agent may be at least 10% of the recommended dose.

EXAMPLE

A comparative study was performed to show the synergistic effect obtained using the 2-methoxy-substituted alkylglycerols in combination with cytostatic agents in chemotherapeutic treatment of cancer.

Subject recruitment and selection: Ten (10) patients were studied. A biopsy was taken from each and analyzed using in vitro cultures.

Method - Fluorescent cytoprint assay: The fluorescent cytoprint in vitro assay was designed to measure the effectiveness of specific chemotherapy drugs in destroying individual patients' cancer cells. Tumour tissue samples were "sandwiched" between two thin papers coated with collagen and supported by small grids at the surface of the culture medium. This technique assured the tumour samples, called "micro-organs"

(300-500 viable tumour cells having the same structure and function of the original tumour) would be stationary and could be monitored over time under the microscope and photographed. The tissue samples were then exposed to a panel of chemotherapeutic agents and examined to see how many and which micro-organs had been killed. Drugs were also tested in varying concentrations.

Shipping and handling of specimen sample:
Each specimen was placed in a tube with transport medium and shipped overnight in a freezer pack to Analytical Biosystems. Upon arrival the specimen was transferred to a laminar flow hood for processing and assay. A sample of 1 gram of viable tumour tissue was sufficient for assay of the treatments at three different concentrations. The specimen was centrifuged, washed with fresh medium, and after mincing, collagenase was added. The culture was then incubated for 18-24 hours.

Culture set up:
Following the initial incubation, the micro-organ cultures were prepared. Tumour fragments were collected by centrifugation, washed, and resuspended in media. After 30 minutes in the dark, large fragments (100-1500 cells) were planted in a matrix of cellulosa fibers impregnated with collagen. These micro-organ cultures were placed on stainless steel screen supports located in each well of a 24-well tissue culture plate. Medium was added so that the culture sat at the liquid gas interface and was fed by capillary action through the cellulose matrix. Cultures were returned to the incubator for 24 hours.

Fluorescent cytoprinting:
Following the 4 hours incubation, the initial cytoprint was prepared. Fluorescein acetate in serum-free medium was added. Viable tumour cell clusters or micro-organs with intact cell membranes retained fluorescein released from the substrate and became fluorescent. After 30 minutes in the dark, cultures

were washed and the patterns of fluorescent micro-organs (cytoprints) were recorded photographically under a dissecting microscope. This record served as the baseline, i.e., each culture served as its own baseline when cytoprinting was repeated at the end of the assay period. Cultures were then returned to the medium to allow viable tumour cell clusters to expel the fluorescein.

Drug Treatment

The chemotherapeutic drugs employed were Doxorubicin (Adriamycin®) and Fluorouracil. They have been dosed to give a concentration in the cultured tumour cells similar to the concentration of these drugs in patients being chemotherapeutically treated with these drugs.

In addition to these drugs, lipid-based emulsions of two types were used: one the one hand consisting of a mixture of alkylglycerols as extracted from shark liver oil, on the other hand consisting only of 1-O-(2-methoxy)hexadecylglycerol extracted from shark liver oil. The concentrations of the latter were 10^{-5} molar, corresponding to 3.46 microgram per milliliter and 10^{-6} molar corresponding to 0.346 microgram per milliliter.

In the initial studies we determined tumour susceptibility following:
(a) the addition of lipid-based alkylglycerol emulsions, (b) chemotherapeutic agents, and (c) lipid-based alkylglycerol emulsions plus chemotherapeutic agents. The drug groups were run concurrently within any one assay. All samples including control (no drug) were carried out in duplicate.

Evaluation and Drug Effects:

Cytotoxicity (loss of fluorescent micro-organs) was assessed by comparing photographic and fluorescent cytoprints taken before and after treatment. Results of the cytotoxicity was reported as "sensitive" (greater than 90% cell death); "intermediate" (between 25 and 90% cell death), and "resistant"

(less than 25% cell death). Tumour growth and viability was indicated by comparing changes in shape and size of the micro-organs following drug treatment with the initial cytoprints of untreated cultures of the same specimen (control).

The results are illustrated in the accompanying figure showing the percentage of killed tumour cells using either the alkyl-glycerol alone, the alkylglycerol in association with the cytotoxic agent and the cytotoxic agent alone, respectively.

This study thus allowed us to examine the effects of alkyl-glycerols on the viability and susceptibility of breast cancer cells and to determine whether they can serve as an adjuvant to chemotherapeutic agents.

These results thus revealed the following: Out of ten patients who received a combination of chemotherapy and alkyl-glycerols, six tests resulted in tumour sensitivities at, or above, 90%, compared with only one patient in the group receiving doxorubicin or fluorouracil alone.

In our study group, one patient's tumour tissue sample was inadequate and a test combining doxorubicin, fluorouracil and alkylglycerol was not possible. This patient was also the only individual that showed sensitivity to chemotherapy alone in all groups tested.

Of the nine remaining patients six had sensitivities of 90% or better. In the three patients who did not fit this criteria one patient showed a sensitivity at or above 80% when alkyl-glycerols were combined with the chemotheapeutic agents, and in the two patients remaining both showed an increased sensitivity to chemotherapy when alkylglycerols were used.

The patients possessed the following characteristics: Six patients had breast cancer, one had metastatic adenocarcinoma of the lung, one had mesothelioma, one had colon cancer, and

one had renal cancer.

In the breast cancer group five of the patients in this group had infiltrating ductal carinoma, and one had adenocarcinoma. Three of the patients with infiltrating ductal carcinoma reached sensitivity levels at or above 90% when chemotherapy was used in combination with alkylglycerols. Another patient had an improvement in their sensitivity from resistant to intermediate, and in one case there was no improvement noted. The patient with adenocarcinoma in this group had an inadequate tissue sample, and we were unable to compare results. One of these patients was also tested with a specific fraction of alkylglycerol, the 2-methoxyglycerol 1-O-(2-methoxy)hexadecylglycerol. When this compound was added to one of the tumour cultures in combination with doxorubicin the highest response rate was seen, and the tumour went from approximately 90% sensitivity to greater than 90% sensitivity. With fluorouracil alone, the tumour was resistant, and exhibited intermediate sensitivity when used in combination with the 2-methoxyglycerol.

In the mesothelioma patient the sample was resistant to all chemotherapeutic agents when given alone. When the tumour was exposed to a combination of doxorubicin and alkylglycerol the tumour response was at 90%.

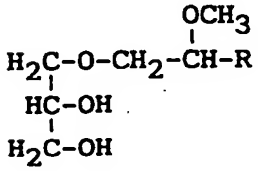
The second lung cancer patient sample was a metastatic lesion from a primary colon cancer. This sample was resistant to doxorubicin and showed an intermediate sensitivity to fluorouracil. When exposed to alkylglycerols the sensitivity increased to intermediate level i combination with doxorubicin, and further increased to sensitive in combination with fluorouracil. This was found to be the case in both the mid dose and the high dose groups.

In the clear cell-predominant renal cell carcinoma patient the tumour showed an intermediate sensitivity to doxorubicin and

fluorouracil, and was resistant to an additional chemo-therapeutic agent, vinblastine. In the mid dose alkylglycerol/-doxorubicin combination, more than 80% of the tumour was killed. However, this did not meet the 90% or better criteria.

CLAIMS

1. A pharmaceutical composition for the treatment of cancer containing as an active ingredient at least one 2-methoxy-substituted alkylglycerol of the formula



wherein R is a hydrogen atom or a straight or branched, saturated or unsaturated alkyl group of from 1 to 24 carbon atoms; c h a r a c t e r i z e d in that it also contains at least one cytostatically active agent.

2. A pharmaceutical composition according to claim 1 c h a r a c t e r i z e d in that the 2-methoxy-substituted alkylglycerol is derived from shark liver oil.

3. A pharmaceutical composition according to claim 1 or 2 c h a r a c t e r i z e d in that the 2-methoxy-substituted alkylglycerol is selected from 1-O-(2-methoxy)hexadecylglycerol (16:0), 1-O-(2-methoxy)-4-hexadecenylglycerol (16:1) and the 1-O-(2-methoxy)-4-octadecenylglycerol (18:1).

4. A pharmaceutical composition according to any of the above claims c h a r a c t e r i z e d in that the alkylglycerol and the cytostatically active agent are present in a molar ratio to each other of from 1:100 to 100:1.

5. A pharmaceutical composition according to claim 4 c h a r a c t e r i z e d in that the alkylglycerol and the cytostatically active agent are present in a molar ratio to each other of from 1:20 to 20:1

6. A pharmaceutical composition according to claim 5 c h a r a c t e r i z e d in that the alkylglycerol and the cytostatically active agent are present in a molar ratio to each other of from 1:10 to 10:1.

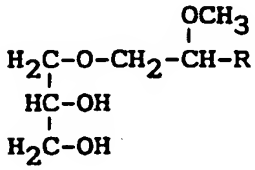
7. A pharmaceutical composition according to claim 6 c h a r a c t e r i z e d in that the alkylglycerol and the cytostatically active agent are present in a molar ratio to each other of from 1:5 to 5:1

8. A pharmaceutical composition according to claim 7 c h a r a c t e r i z e d in that the alkylglycerol and the cytostatically active agent are present in a molar ratio to each other of from 1:2 to 2:1.

9. A pharmaceutical composition according to any of the above claims c h a r a c t e r i z e d by being an lipid-based solution or emulsion.

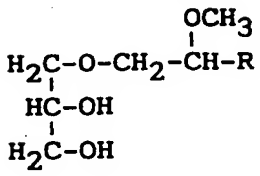
10. A pharmaceutical composition according to any of the claims 1-8 c h a r a c t e r i z e d by being in the form of micellas in an oily vehicle.

11. Use of a 2-methoxy-substituted alkylglycerol of the formula



wherein R is a hydrogen atom or a straight or branched, saturated or unsaturated alkyl group of from 1 to 24 carbon atoms; in association with a cytostatically active agent, in preparing a pharmaceutical composition according to any of the claims 1-10.

12. Use of a 2-methoxy-substituted alkylglycerol according to the formula



wherein R is a hydrogen atom or a straight or branched, saturated or unsaturated alkyl group of from 1 to 24 carbon atoms in preparing a pharmaceutical composition to be used in conjunction with a cytostatically active agent in the treatment of cancers to synergistically enhance the efficiency and specificity of said cytostatically active agent.

13. Use according to claim 12 c h a r a c t e r i z e d in that the 2-methoxy-substituted alkylglycerol is derived from shark liver oil.

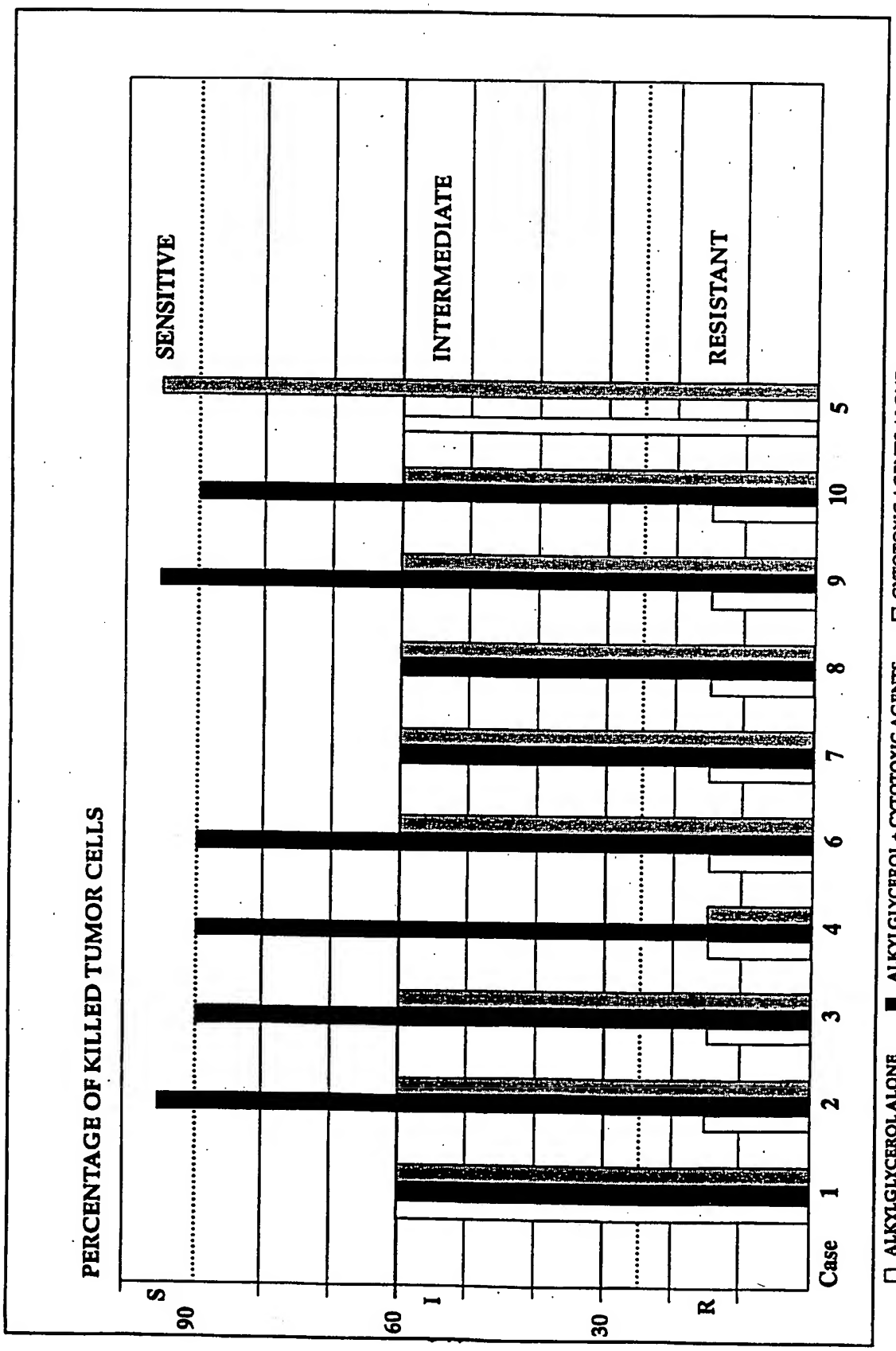
14. Use according to claim 13 c h a r a c t e r i z e d in that the 2-methoxy-substituted alkylglycerol is selected from 1-O-(2-methoxy)hexadecylglycerol (16:0), 1-O-(2-methoxy)-4-hexadecenylglycerol (16:1) and the 1-O-(2-methoxy)-4-octadecenylglycerol (18:1).

15. A method of chemotherapeutic treatment of cancer by the administration of a composition according to any of claims 1-10.

16. A method of chemotherapeutic treatment of cancer by the administration of a composition obtained by the use of a 2-methoxy-substituted alkylglycerol according to claim 11.

17. A method of chemotherapeutic treatment of cancer by the administration of a composition obtained by the use of a 2-methoxy-substituted alkylglycerol according to any of claims 12-14, c h a r a c t e r i z e d in that said composition is administered in conjunction with a cytostatic agent.

18. A method according to any of claims 15-17 c h a r a c t e r i z e d in that the 2-methoxysubstituted alkylglycerol is administered in a daily dosage of 3 -30 mg and the cytostatic agent is administered in a daily dosage of at least 10% of its recommended daily dose.


PERCENTAGE OF KILLED TUMOR CELLS
SENSITIVE
INTERMEDIATE
RESISTANT
S
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60
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8
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5
ALKYLGLYCEROL ALONE
ALKYLGLYCEROL + CYTOTOXIC AGENTS
CYTOTOXIC AGENTS ALONE

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00954

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/075
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	US 4046914 A (B.E. HALLGREN ET AL), 6 Sept 1977 (06.09.77) --	1-14
Y	GB 1194238 A (ASTRA NUTRITION AB), 10 June 1970 (10.06.70) --	1-14
A	US 3432602 A (A.H. BROHULT ET AL), 11 March 1969 (11.03.69) -- --------	1-14

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 August 1998	31.08.98
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Eva Johansson Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.
PCT/SE 98/00954

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